Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713409232

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Online Publication Date: 01 January 2008

To cite this Article Wright, S.A. Wineriter and Center, T. D.(2008)'Nonselective oviposition by a fastidious insect: the laboratory host range of the melaleuca gall midge Lophodiplosis trifida (Diptera: Cecidomyiidae)',Biocontrol Science and Technology,18:8,793 — 807
To link to this Article: DOI: 10.1080/09583150802344902
URL: http://dx.doi.org/10.1080/09583150802344902

Biocontrol Science and Technology,
Vol. 18, No. 8, 2008, 793–807

Taylor & Francis
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Nonselective oviposition by a fastidious insect: the laboratory host range of the melaleuca gall midge *Lophodiplosis trifida* (Diptera: Cecidomyiidae)

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(Received 4 March 2008; returned 29 April 2008; final version received 14 July 2008)

Invasion by the Australian paperbark, *Melaleuca quinquenervia*, has degraded large areas of south Florida wetlands. Restoration of these wetlands requires the removal of expansive monocultures of this large tree while simultaneously curtailing its spread. Management strategies developed by federal and state agencies include biological control to halt the spread of this species and to prevent reinfestation of cleared areas. This requires biological agents able to reduce flowering, seed production, and growth while increasing mortality of seedlings and saplings. Two of the three introduced agents (*Oxyops vitiosa* Pascoe and *Boreioglycaspis melaleucae* Moore) partially meet these needs but outcomes are not spatially or temporally consistent. Thus, additional agents are needed. The bud-gall fly *Fergusonina turneri* Taylor, with its mutualistic nematode *Fergusobia quinquenerviae* Davies and Giblin-Davis, is actively being released but has not established. A fourth promising agent, the gall midge *Lophodiplosis trifida* Gagné, manifested an extremely narrow host range during laboratory testing. Oviposition was indiscriminant in caged environments. Small, incipient, unilocular galls were initiated on *Melaleuca viminalis*, but larval development ensued only on *M. quinquenervia*. The unilocular galls on *M. viminalis* did not grow and produced no adult flies. As a result, *M. viminalis* test plants suffered only minor cosmetic damage. Observations from both Australia and Florida attest to the ability of this midge to impede *M. quinquenervia* growth and kill small plants. Thus, *L. trifida* is safe to release and will likely contribute to management objectives for control of this pernicious wetland invader.

Keywords: Cecidomyiidae; Everglades; host range; *Lophodiplosis trifida*; *Melaleuca quinquenervia*; weed biocontrol

Introduction

The Australian broad-leaved paperbark tree, *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae), commonly called 'melaleuca', was intentionally introduced into Florida for ornamental, soil stabilisation, and agroforestry purposes prior to 1906 (Turner, Center, Burrows, and Buckingham 1998; Serbesoff-King 2003; Dray, Bennett, and Center 2006). It was widely planted in wetlands as an inexpensive production method for the nursery trade and in an attempt to produce timber, a harvestable commodity. As a result, this exotic tree naturalised and over time displaced much of the native vegetation as it invaded the diverse wetland habitats of the Florida Everglades (Turner et al. 1998). *Melaleuca quinquenervia*,

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ISSN 0958-3157 print/ISSN 1360-0478 online

DOI: 10.1080/09583150802344902
http://www.informaworld.com

recognised as an invasive weed during the late 1970s, was placed on the Florida Prohibited Plant List in 1990, and was added to the Federal Noxious Weed List during 1992.

Lophodiplosis trifida Gagné (Diptera: Cecidomyiidae), a gall midge, was originally reported as an inquiline (Gagné, Balciunas, and Burrows 1997). It was thought to occupy galls actually caused by three other *Lophodiplosis* spp. on members of the broad-leaved *Melaleuca leucadendra* species complex in Queensland and New South Wales, Australia. We now know that *L. trifida* directly utilises these species, forming galls on *M. dealbata* S.T. Blake, *M. quinquenervia*, and *M. viridiflora* Sol. ex Gaertn. (Purcell, Winewriter, and Brown 2007) in the field as well as *M. argentea* W.V. Fitzg. and *M. cajuputi* Powell under laboratory conditions. All of these host species are closely related, being taxonomically circumscribed within the *Melaleuca leucadendra* complex.

Lophodiplosis trifida females oviposit eggs on surfaces of stems, leaves, and buds. Because eggs are loosely attached to plant surfaces, galling (Figure 1b) is likely induced by larval secretions rather than oviposition as in some *Asphondylia* spp. (Gagné 1989). Stem and bud galls disrupt apical and axillary meristem growth, preempting flowering and subsequent seed production.

The holotype *L. trifida* specimen was collected from a blister leaf gall on *Melaleuca quinquenervia* during 1995 (Gagné et al. 1997). Additional specimens were collected by J.K. Balciunas and D.W. Burrows while surveying seven *Melaleuca* spp. of the *Melaleuca leucadendra* complex (Gagné et al. 1997). Gagné et al. (1997) described this species, placing it in a new genus, *Lophodiplosis*. The species *L. trifida* is readily identified by a diagnostic three-pointed projection at the vertex of the pupa. Holotype and paratype *L. trifida* specimens have been deposited in the Australian National Insect Collection, Canberra, Australia. Additional specimens from Australia are lodged in the Museum of Natural History, Washington, DC.

We hypothesised that the host range of *L. trifida* was restricted to a few *Melaleuca* species in the broad-leaved *M. leucadendra* species complex. To test this hypothesis, host range studies of *L. trifida* were conducted first in Australia (Australian Biological Control Laboratory 2002) and then more extensively under quarantine conditions in Florida, USA. It was considered a candidate biological control agent because of its seemingly restricted

Figure 1. (a) A young *Melaleuca quinquenervia* stem. (b) A *M. quinquenervia* stem 6 weeks after exposure to *Lophodiplosis trifida*, with stem, buds, and leaves galled. The galled tissue is undergoing lignification and new growth is evident. A midge is resting on the right side of the left gall just below a leaf, and many pupal exuviae, left behind after eclosion, are projecting from the gall surfaces. (c) A young *Melaleuca viminalis* stem. (d) A *M. viminalis* stem 6 weeks after exposure to *L. trifida*, swollen at the tip. No lignification is apparent and new leaves at the apex indicate continued growth. (e) A visual comparison of cleared galled tissues of *M. quinquenervia* (left) and *M. viminalis* (right) in which *L. trifida* had the opportunity to develop for 6 weeks. Many tightly packed, mature galls are apparent in *M. quinquenervia* tissue while scattered incipient, unilocular, failed galls are present in *M. viminalis* tissue, clearly illustrating its inability to develop to maturity on *M. viminalis*. (f) Comparison of a *L. trifida* first instar larva collected as it penetrated *M. quinquenervia* bud tissue (top), an *L. trifida* last instar larva dissected from a 6-week-old *M. quinquenervia* gall (centre), and a typical size larva dissected from a 6-week-old *M. viminal*is gall (right). (g) Four branches of a test plant, *Tibouchina granulosa*, caged for oviposition and development tests. (h) Two *M. quinquenervia* plants selected for a damage study (top) and a comparison of their growth after 5 months (bottom). The plant on the left was not exposed to *L. trifida*, whereas the one on the right was exposed to 15 females at the start of the study. Even though F_1 adults were removed every 24 h, some apparently were not found so that the plant was repeatedly attacked and galled.

host usage, its ease of colonisation in the laboratory, and the significant damage to *M. quinquenervia* plants observed in Australia.

Florida host range studies were conducted at a quarantine facility located in Gainesville. Biology studies, which were needed to support host range determinations, were conducted concurrently and will be reported separately.

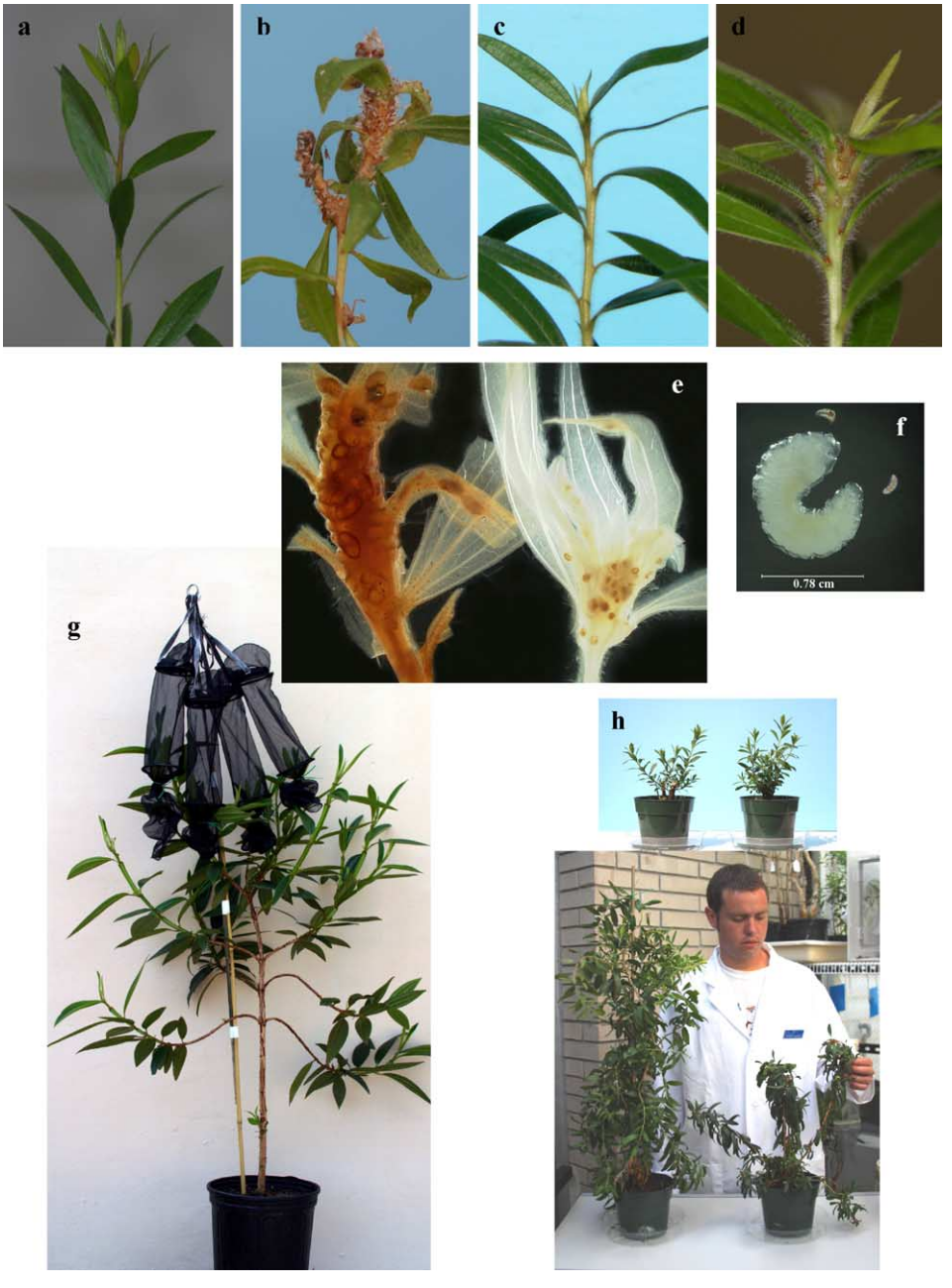

Figure 1 (Continued)

Materials and methods

Host range determination of *L. trifida* was based on 64 test plant species (Table 1), 40 of which, including the target weed *M. quinquenervia*, were species of Myrtaceae and 24 of which were species from 21 other plant families. The *L. trifida* used in the tests were reared from three shipments of bare-rooted, galled *M. quinquenervia* seedlings collected in Australia. Fifteen galled plants were received on 9 October 2003, 30 on 17 October 2003, and 33 on 29 January 2004. This colony was derived from infested *M. quinquenervia* trees surrounding the CSIRO Long Pocket Laboratories located at Indooroopilly, Queensland (S27°30.668' E152°59.838').

Most test plants were purchased from Florida nurseries or grown from seeds or cuttings obtained from nursery-grown or field-collected plants. Seeds of *Leptospermum lanigerum*, *L. petersonii*, *L. rotundifolium*, *Melaleuca alternifolia*, *M. armillaris*, and *M. trichostachya* were imported under permit from Australia or purchased from B & T World Seeds, France, and cultivated outdoors at the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, where our Gainesville lab is located. Small plants of a few species were transplanted, with permission, from natural habitats in Florida. *Melaleuca quinquenervia* plants were grown from seed obtained from naturally occurring infestations in south Florida. Plants tested ranged from 0.25 to 2 m in height.

Potted test plants were held outdoors until being transferred to the quarantine greenhouse for testing. Most plants tested were shrub-like and primarily woody in nature. They were routinely fertilised and pruned to induce new growth, which provided optimal tissue for gall formation.

Preliminary oviposition tests on cuttings of both myrtaceous and non-myrtaceous species proved to be of no value for determining host range due to indiscriminate oviposition by *L. trifida* females. This behaviour mandated testing protocols designed to determine whether *L. trifida* would induce galls and fully develop on nontarget species. Formation of galls depends on the induction of cell hypertrophy of living plant tissue so only whole, living plants were used for development tests.

Plants were tested in groups: 117 groups included three nontarget plants and a *M. quinquenervia* plant; three groups included two nontarget plants and a *M. quinquenervia* plant. Pruned plants produced new growth asynchronously so groups were not replicated *per se* although test species were replicated as members of different groups. For example, *Vitis rotundifolia* was included in a group with *M. quinquenervia*, *Ilex cassine*, *Leptospermum rotundifolium*, then in another group with *M. quinquenervia*, *Pittosporum tobira*, and *Syzygium samarangense*.

Our goal was to test five plants per species, with each plant considered a replicate. In reality one to two plants were tested for eight species, three to four plants for 13 species, and five to eight plants for 37 species (Table 2). Fewer than five plants were tested when insufficient numbers were available, when controls produced too few or no galls (15 of 120 tests), and when plants died or were damaged during testing (25 plants). More plants were tested when gall development on the controls was not obvious early in the test period. Setting up additional tests seemed more efficient than waiting 6 weeks until the former tests were completed.

No-choice host range tests

Two no-choice tests were simultaneously conducted on each plant: an oviposition test and a development test. Even though oviposition tests were of limited value in predicting host

Table 1. Test plant list used to determine host range of *Lophodiplosis trifida*[abc].

Genus and species[df]	Common name[ef]	N American status[g]
Wapshere Category[h] 1 – Genetic type of the target weed species		
Melaleuca quinquenervia (Cav.)S.T.Blake	melaleuca, paperbark, punk	FL invasive exotic; CA, HI, LA, PR exotic
Wapshere Category 2 – Same genus as target weed		
Melaleuca alternifolia Maiden & Betche ex Cheel	narrow leaved tea tree	not present
Melaleuca armillaris (Sol. ex Gaertn.)Sm.	bracelet or giant honey myrtle	CA exotic
Melaleuca citrinus (Curtis)Skeels (as 'citrina')	crimson bottlebrush	FL, LA, PR exotic
Melaleuca viminalis (Sol. ex Gaertn.)Byrnes	weeping bottlebrush	FL naturalised exotic; CA exotic
Melaleuca trichostachya Lindl.		FL exotic, CA exotic?
Wapshere Category 3 – Species in other genera in the same family and subfamily, Leptospermoideae, as target weed		
Eucalyptus amplifolia Naudin	cabbage gum	FL exotic
Eucalyptus camaldulensis Dehnh.	Murray red gum	CA, HI, PR exotic
Eucalyptus cinerea F. Muell. ex Benth.	silver dollar tree	HI exotic
Eucalyptus grandis W.Hill	rose gum	FL exotic
Leptospermum lanigerum (Sol. ex Aiton)Sm.	woolly tea tree	CA, FL exotic
Leptospermum petersonii F.M.Bailey (as '*Petersoni*')	lemon scented tea tree	CA, HI exotic
Leptospermum rotundifolium Domin [nom. illeg.]	round-leaved tea tree	CA exotic
Leptospermum scoparium J.R. Forst. & G. Forst.	manuka or manuka tea tree	FL, HI exotic
Wapshere Category 3 – Species in other genera in the same family as target weed, subfamily Myrtoideae		
Acca sellowiana (O. Berg)Burret	feijoa, pineapple guava	FL exotic and crop
Calyptranthes pallens Griseb.	pale lidflower, spicewood	FL native
Calyptranthes zuzygium (L.)Sw.	myrtle-of-the-river	FL native
Eugenia aggregata (Vell.)Kiaerskov.	cherry-of-the-Rio-Grande	FL exotic
Eugenia axillaris (Sw.)Willd.	white stopper	FL native
Eugenia brasiliensis Lam.	Brazil cherry	FL exotic
Eugenia confusa DC.	redberry stopper; redberry Eugenia	FL native
Eugenia foetida Pers.	Spanish stopper, boxleaf stopper	FL native
Eugenia reinwardtiana (Blume)DC.	mountain stopper	FL exotic
Eugenia rhombea Krug & Urb. ex Urb.	red stopper	FL native
Eugenia uniflora L.	Surinam cherry	FL naturalised exotic
Eugenia uvalha Camb.	uvalha	FL exotic
Mosiera longipes (O. Berg)Small	mangroveberry	FL native
Myrcianthes fragrans (Sw.)McVaugh	twinberry, Simpson's stopper	FL native
Myrciaria cauliflora (C.Martius)O.Berg	jaboticaba	FL exotic crop
Pseudanamomis umbellulifera (Kunth)Kausel		FL exotic
Pimenta dioica (L.)Merr.	allspice, pimento	FL exotic
Pimenta racemosa (Mill.)J.Moore	bay rum tree	FL exotic

Table 1 (*Continued*)

Genus and species[df]	Common name[ef]	N American status[g]
Psidium cattleianum Sabine	strawberry guava	FL invasive exotic
Psidium friedrichsthalianum (O. Berg)Niedenzu	Costa Rican guava	FL exotic
Psidium guajava L.	guava	FL naturalised exotic, crop
Syzygium cumini (L.)Skeels	Java plum	FL invasive exotic
Syzygium jambos (L.)Alston	Malabar plum, rose apple	FL exotic
Syzygium malaccense (L.)Merr. & Perry	rose or malay apple	FL exotic
Syzygium paniculatum Gaertn. (*E. compacta*)	Australian brush cherry	FL exotic
Syzygium samarangense (Blume)Merr. & Perry	wax jambu	FL exotic, crop
Wapshere Category 4 – Threatened and endangered species in the same family as target weed		
Calyptranthes pallens Griseb.	pale lidflower, spicewood	FL threatened
Calyptranthes zuzygium (L.)Sw.	myrtle-of-the-river	FL endangered
Eugenia confusa DC.	redberry stopper, redberry Eugenia	FL endangered
Eugenia rhombea Krug & Urb. ex Urb.	red stopper	FL endangered
Mosiera longipes (O. Berg)Small	mangroveberry	FL threatened
Myrcianthes fragrans (Sw.)McVaugh	Simpson's stopper	FL threatened
Wapshere Category 5 – Species in the same order (myrtales) as target plant Order, Family, Genus and Species		
Melastomataceae: *Tibouchina granulosa* (Desr.)Cogn.	glory bush	FL exotic
Combretaceae: *Bucida buceras* L.	black olive	FL exotic
Lythraceae: *Lagerstroemia indica* L.	crapemyrtle	FL exotic
Wapshere Category 6 – Species in other orders than the target weed		
Laurales: Lauraceae: *Persea americana* Mill	avocado	FL exotic, crop
Urticales: Moraceae: *Ficus aurea* Nutt.	golden fig, strangler fig	FL native
Myricales: Myricaceae: *Myrica cerifera* L.	southern bayberry, wax myrtle	FL native
Fagales: Fagaceae: *Quercus virginiana* Mill.	live oak	FL native
Theales: Clusiaceae: *Hypericum fasciculatum* Lam.	sandweed, peelbark St. John's-wort	FL native
Salicales: Salicaceae: *Salix carolinina* Michx.	Carolina willow, coastalplain willow	FL native
Evenales: Sapotaceae: *Sideroxylon reclinatum* Michx.	Florida bully	FL native
Primulales: Myrsinaceae: *Rapanea punctata* (Lam.)Lundell	myrsine, colicwood	FL native
Rosales: Pittosporaceae: *Pittosporum tobira* (Thunb.)Aiton	Japanese cheesewood	FL exotic
Rosales: Rosaceae: *Eriobotrya japonica* (Thunb.)Lindl.	loquat	FL exotic
Rosales: Rosaceae: *Prunus caroliniana* (Mill.)Aiton	Carolina laurelcherry	FL native
Celastrales: Aquifoliaceae: *Ilex cassine* L.	Dahoon	FL native
Rhamnales: Vitaceae: *Vitis rotundifolia* Michx.	Muscadine	FL native

Table 1 (*Continued*)

Genus and species[df]	Common name[ef]	N American status[g]
Sapindales: Rutaceae: *Citrus* x *limon* (L.)Osbeck	lemon	FL exotic, crop
Sapindales: Rutaceae: *Citrus* x *aurantium* L.	grapefruit	FL exotic, crop
Sapindales: Rutaceae: *Citrus* x *aurantium* L.	sweet orange	FL exotic, crop
Dipsacales: Adoxaceae: *Sambucus nigra* L. supbsp. *Canadensis* (L.)Bolli	America elder, elderberry	FL native
Arecales: Arecaceae: *Serenoa repens* (w. Bartram)Small	saw palmetto	FL native
Cyperales: Poaceae: *Saccharum officinarum* L.	sugarcane	FL exotic, crop
Cupressales: Cupressaceae: *Taxodium distichum* (L.)Rich.[b]	bald-cypress	FL native
Pinales: Pinaceae: *Pinus elliottii* Englem.[b]	slash pine	FL native

[a]Except where noted all plant species listed are angiosperms. [b]Angiosperm family and Myrtaceae subfamily classification according to Cronquist (1981). [c]Gymnosperm family classification according to Farjon (1998). [d]Scientific names of native Australian plants were taken from the Australian Plant Name Index. [e]Common names of native Australian plants were taken from the Australian Plant Common Name Database. [f]Scientific and common names of USA plants were taken from Mabberley (1997) and Wunderlin and Hansen (2004). [g]Status terms of exotic, naturalised exotic, and invasive naturalised exotic are designations used by Brown (2006). Wunderlin and Hansen (2004) was used to determine native status in Florida. Hickman (1993) and The PLANTS Database were used to determine status in other USA states. Coile and Garland (2003) was used to determine status of Florida's native endangered and threatened plants. [h]The categories of the test plant list are based on Wapshere (1974).

range, they provided rapid feedback on whether the control for the development test of each group would be positive after 6 weeks, i.e. branches would be galled and eclosion underway. Also, if excessive oviposition occurred on some nontarget species, this would suggest the possibility of discriminant oviposition behaviour with potential development. Oviposition tests were therefore conducted simultaneously with all but the first 10 of 120 development tests.

Each test group was set up as follows. Plants of three non-target species and one *Melaleuca quinquenervia* plant, each with at least four branches bearing new growth, were

Table 2. Number of replicates/plant species used in *Lophodiplosis trifida* no-choice oviposition and gall development tests.

Species (total attempts to test if <5 replicates)	No. of replicates
Eugenia brasiliensis(5), *Pseudanamomis umbellulifera*(1)	1
Eugenia aggregata(3), *E. uvalha, Leptospermum lanigerum*(3), *L. rotundifolium*(4), *Sideroxylon reclinatum, Syzygium malaccense*	2
Calyptranthes pallens(5), *Eucalyptus amplifolia*(6), *Eugenia axillaris*(6), *Melaleuca trichostachya*(5), *Syzygium jambos*(5)	3
Melaleuca citrinus(6), *Eucalyptus cinerea*(5), *Eugenia foetida*(6), *E. uniflora*(6), *Ilex cassine*(5), *Lagerstroemia indica*(5), *Leptospermum petersonii*(5), *Melaleuca alternifolia*(6), *M. armillaris*(6), *Psidium cattleianum*(7), *Serenoa repens*(5), *Taxodium distichum, Tibouchina granulosa*	4
All other species ($N = 28$, excludes *M. quinquenervia*)	5
Leptospermum scoparium, Salix caroliniana, Sambucus nigra, Vitis rotundifolia	6
Melaleuca viminalis, Mosiera longipes, Pimenta dioica, Syzygium cumini	7
Eucalyptus camaldulensis	8

selected from outdoor growing areas. When a group was assembled, it was assigned a unique identifying number. Each of the four branches bearing new growth on each plant was then enclosed in a sleeve cage (10 × 30 cm, w × l, with a zipper at the top) (Figure 1g). If there were differences in the number of new shoots among the four caged branches, the three caged branches having the most new shoots were assigned to the 6-week development test, and the caged branch having the least new shoots to the 4–5-day oviposition test (4–5 days). This procedure was not possible for four plants that were too small. In these cases, most branches were enclosed in one sleeve cage for a development test and a single or few branches enclosed in a second cage for the oviposition test.

Lophodiplosis trifida adults from stock colonies, aspirated in groups of two males and five females, were randomly distributed among all test plant species, first to the three development test cages on each plant and then to the oviposition cage on each plant. A second allocation of adults was added the next day following the same procedure. Adults were added in two rounds because of limited availability due to skewed sex ratios in colonies and asynchronous emergence. Occasionally, insufficient adults were available to complete one round per day, so the balance needed/cage was added on a third day. In total, four males and 10 females were caged on each plant for the oviposition test and 12 males and 30 females were caged on each plant for the development test. If five plants per species were tested, 50 *L. trifida* females could oviposit on each plant species and 150 females had the opportunity to produce offspring on each plant species.

The branches of some nontarget species did not grow well within sleeve cages during the 6-week test period for development. We removed cages from sensitive plants after all adults had died. We intended to cage them again if galls developed, but none did. *M. quinquenervia* controls were caged throughout the test period.

Evaluation of oviposition tests

Under our quarantine greenhouse conditions (24°C, 55–74% RH, 16 h L:8 h D) adult cecids lived about 2 days and eggs began hatching after about 6 days. Therefore, the plant material caged for oviposition was cut from all plants in the group 4–5 days after test initiation, and then carefully examined to count eggs. If eggs were not observed on the control branch ($N = 5$ tests of 110), either the test was not repeated (Test groups 11 and 69), the test was repeated with the same control (Test group 62), or with a new control (Test groups 68 and 74). When the oviposition tests were repeated (62, 68 and 74), a new set of adults (4 males: 10 females) was added to all oviposition and corresponding development cages. For test group 69, branches of the development test control were examined *in situ* and two of three branches had eggs so no additional adults were added to either the oviposition or development cages. (Note: In the end, development data from Test groups 11, 62 and 68 were eliminated from the results because there were too few galls on the controls.)

Macroscopic evaluation of development tests

The caged plant material from the development tests was cut 6 weeks after the start of the test, and examined using a ×5 jeweller's headset to search for galls or obvious damage. The presence of a new generation of adults, evident from pupal exuviae left loosely attached to the galls after eclosion (Figure 1b) or from the presence of live adults,

was recorded. The material was then cleared and softened for later dissection using Nesbitt's solution.

Microscopic evaluation of development tests

Cleared plant material was dissected to search for galls through a stereomicroscope at a magnification range of $\times 60$–250. Tissues where galls had been initiated but had not fully formed were teased apart to dislodge any larvae present; tissues where galls were more advanced or fully mature were cut open to release larger larvae or pupae, or to reveal empty, eclosed gall chambers. Developmental stages of *L. trifida* present in galls (or absent due to eclosion) were tallied when galls were found on nontarget plants in a test group. If none were found, those on the control were counted until 30 galls had been tallied per cage. If fewer than 15 galls were on the control, the test was considered invalid, and new plants, if available were tested. Fifteen galls was selected as the cut-off point because when results of controls were ranked in ascending order, there was a natural break in the data. Data were also disregarded if a plant was damaged or had died. However, non-target test species were examined for gall development even though the control may have failed or if the test plant had died or was damaged.

Analysis of data

Analysis of oviposition data

The consistency of controls in oviposition tests was measured by calculating the percent *M. quinquenervia* plants oviposited on in all tests. This percent was compared with the percent on all other species combined. For each species, including the target plant, the mean number of eggs laid $\pm$SE was determined. The *t*-test test was used to determine whether differences between the means of each plant species and the corresponding *M. quinquenervia* controls were significant.

Analysis of development test macroscopic data

The consistency of controls in development tests was measured by comparing the number of galled *M. quinquenervia* plants with those not galled, and by determining the percent plants with 0–3 stems galled. For those nontarget species where partial or complete development occurred, percent plants galled/species and percent plants/species with 0–3 stems galled was determined and compared with the corresponding *M. quinquenervia* controls. Chi-square analysis was used to determine whether differences were significant.

Analysis of microscopic data

For those nontarget species where partial or complete development occurred, the mean number of gall chambers/plant/species $\pm$SE was determined. The *t*-test was used to determine whether differences between the means of each plant species and the corresponding *M. quinquenervia* controls were significant.

Results and discussion

Oviposition tests

Lophodiplosis trifida oviposited on 96% of the *M. quinquenervia* controls (110 plants) in no-choice oviposition tests on potted plants. Females laid an average of 269.6 ± 15.44 eggs/plant (±SE, range 0–676) on these controls. *Lophodiplosis trifida* oviposited on 48 of the 63 nontarget species (76%) but generally laid fewer eggs than on controls (Table 3). However, egg counts on *Myrcianthes fragrans*, *Melaleuca viminalis*, *Eugenia foetida*, and *E. aggregata* were not statistically different from the controls (the latter two due to large variation in the controls). The majority (85%) of eggs oviposited on nontarget species were on five species in three families: *Myrcianthes fragrans* (Myrtaceae: Myrtoideae), *Melaleuca viminalis* (Myrtaceae: Leptospermoideae), *Lagerstroemia indica* (Lythraceae), *Psidium cattleianum* (Myrtaceae: Myrtoideae), and *Ilex cassine* (Aquifoliaceae) (Figure 2). While *L. trifida* failed to exhibit selective oviposition behaviour, apparently not unusual for otherwise fastidious cecidomyiids according to Larsson and Ekbom (1995), these data indicate that some plant species may have been attractive for reasons other than phylogenetic relatedness. If oviposition behaviour had been fastidious, testing would have terminated. However, the lack of ovipositional discrimination mandated that we investigate larval development and survival as well.

Development tests

Macroscopic observations

Of the 120 development tests conducted, *L. trifida* galled *M. quinquenervia* plants in 117. After 6 weeks, all three caged stems were galled on 99 plants (82.5%); two of the three caged stems were galled on 12 plants (10%); and one stem was galled on six plants (5%). The ability of *L. trifida* to gall *M. quinquenervia* was consistent and dramatic in most instances (e.g. compare Figure 1a,b).

No evidence of gall formation was observed on nontarget plants except on *M. viminalis*. The branch tips of four of seven *M. viminalis* plants (57%) appeared swollen on this species after 6 weeks (e.g. compare Figure 1c,d). Three of four *M. viminalis* plants (43%) had three swollen stems and one plant (14%) had two swollen stems (Table 4). This swelling, which was not observed at the macroscopic level on unexposed stems, was attributed to *L. trifida*. So gall initiation clearly occurred on *M. viminalis*.

Conversely, all *M. quinquenervia* plants tested as controls ($N = 7$) produced obvious mature galls on all caged stems, from which some adults had already emerged (Table 4). The number of *M. quinquenervia* and *M. viminalis* plants on which gall initiation occurred differed significantly ($X^2 = 3.82$, df = 1, $P = 0.05$) as well as the number of stems per plant ($X^2 = 5.60$, df = 1, $P = 0.02$), when the frequency of plants with 0–2 stems attacked was compared with those with three stems attacked.

These results show that *L. trifida* can initiate galls on both *M. viminalis* and *M. quinquenervia* but that there is less galling of *M. viminalis*.

Microscopic observations

Fifteen tests in which *M. quinquenervia* plants produced fewer than 15 galls/plant (failed controls) were rejected, leaving 105 of 120 valid development tests. However, as mentioned previously, all nontarget plant species were examined for *L. trifida* development even

Table 3. Results of no-choice *Lophodiplosis trifida* oviposition tests run concurrently with development tests on potted plants[a].

	Test plant				*Melaleuca quinquenervia*			
Plant species	No. of plants tested	No. of plants with eggs	Total eggs	Mean no. eggs/ plant	No. of plants with eggs	Total eggs	Mean no. eggs/ plant	*t* (*P*)
Melaleuca quinquenervia	110				106	29652	269.6±15.44	
Myrcianthes fragrans	4	4	664	166.0±82.85	4	1357	339.3±85.17	1.46 (0.195)
Melaleuca viminalis	5	5	473	94.6±36.89	5	849	169.8±90.28	0.77 (0.463)
Lagerstroemia indica	5	5	242	48.4±21.65	5	1360	272.0±93.25	2.34 (0.048)
Psidium cattleianum	6	4	164	27.3±11.16	6	1466	244.3±85.32	2.52 (0.030)
Ilex cassine	5	5	109	21.8±9.75	5	1873	367.4±100.88	3.41 (0.009)
Syzygium cumini	4	3	31	7.8±3.88	4	571	142.8±43.61	3.08 (0.022)
Melaleuca citrinus	6	2	29	4.8±1.97	6	1386	231.0±50.06	4.52 (0.001)
Eugenia axillaris	5	3	26	5.2±2.33	5	1230	246.0±66.13	3.64 (0.007)
Eugenia confusa	5	3	15	3.0±1.34	5	1570	314.0±73.20	4.25 (0.003)
Eriobotrya japonica	5	1	14	2.8±1.25	5	1992	398.4±49.66	7.96 (<0.001)
Rapanea punctata	5	1	14	2.8±1.25	5	1584	316.8±52.35	6.00 (<0.001)
Prunus caroliniana	5	3	13	2.6±1.16	5	1316	263.2±96.22	2.71 (0.027)
Psidium friedrichsthalianum	3	1	12	4.0±2.31	3	961	320.3±97.91	3.23 (0.032)
Acca sellowiana	5	1	11	2.2±0.98	5	735	147.0±56.43	2.57 (0.033)
Bucida buceras	5	3	11	2.2±0.98	5	1716	343.2±75.99	4.49 (0.002)
Eugenia reinwardtiana	6	3	11	1.8±0.75	4	1560	260.0±88.81	2.91 (0.016)
Salix caroliniana	6	3	10	1.7±0.68	6	1832	305.3±40.87	7.43 (<0.001)
Sambucus nigra	6	3	10	1.7±0.68	5	1240	206.7±66.55	3.412 (0.008)
Calyptranthes pallens	5	1	8	1.6±0.72	5	1534	306.8±74.98	4.07 (0.004)
Persea americana	5	1	8	1.6±0.72	5	1744	348.8±108.78	3.192 (0.013)
Saccharum officinarum	8	3	7	0.9±0.31	8	1783	222.9±40.27	5.51 (<0.001)
Eucalyptus amplifolia	7	2	6	0.9±0.32	5	1011	144.4±53.91	2.66 (0.029)
Vitus rotundifolia	7	4	6	0.9±0.32	7	2882	411.7±47.09	8.72 (<0.001)
Leptospermum lanigerum	3	1	5	1.7±0.96	3	1031	343.7±31.25	10.9 (<0.001)

Table 3 (*Continued*)

Plant species	Test plant				*Melaleuca quinquenervia*			
	No. of plants tested	No. of plants with eggs	Total eggs	Mean no. eggs/ plant	No. of plants with eggs	Total eggs	Mean no. eggs/ plant	*t* (*P*)
Leptospermum scoparium	6	1	5	0.8 ± 0.34	6	869	144.8 ± 52.24	2.76 (0.020)
Pittosporum tobira	5	1	4	0.8 ± 0.36	5	1498	299.6 ± 84.98	3.52 (0.008)
Serenoa repens	5	1	4	0.8 ± 0.36	5	1275	255.0 ± 83.37	3.05 (0.016)
Citrus × aurantium grapefruit	5	2	3	0.6 ± 0.27	5	2016	403.2 ± 33.89	11.9 (<0.001)
Eucalyptus cinerea	5	1	3	0.6 ± 0.27	5	1249	249.8 ± 76.26	3.27 (0.011)
Eucalyptus grandis	7	1	3	0.4 ± 0.16	7	1666	238.0 ± 45.49	5.22 (<0.001)
Calyptranthes zuzygium	6	1	2	0.3 ± 0.14	5	1177	196.2 ± 59.76	3.28 (0.011)
Citrus × aurantium orange	5	2	2	0.4 ± 0.18	5	2136	427.2 ± 39.82	10.7 (<0.001)
Eugenia foetida	6	1	2	0.3 ± 0.14	6	1069	178.2 ± 85.78	2.07 (0.065)
Melaleuca armillaris	6	1	2	0.3 ± 0.14	5	781	130.2 ± 60.17	2.39 (0.040)
Melaleuca trichostachya	5	1	2	0.4 ± 0.18	5	846	169.2 ± 63.71	2.65 (0.029)
Pimenta dioica	6	1	2	0.3 ± 0.14	6	1706	284.3 ± 66.73	4.26 (0.002)
Pimenta racemosa	6	2	2	0.3 ± 0.14	6	1710	285.0 ± 83.89	3.39 (0.007)
Pinus elliottii	7	1	2	0.3 ± 0.11	7	1799	257.0 ± 57.03	4.50 (<0.001)
Quercus virginiana	5	1	2	0.4 ± 0.18	5	1300	260.0 ± 44.56	5.82 (<0.001)
Eucalyptus camaldulensis	5	1	1	0.2 ± 0.09	5	1719	343. 8 ± 30.07	11.4 (<0.001)
Eugenia aggregata	3	1	1	0.3 ± 0.19	2	300	100.0 ± 94.06	1.422 (0.250)
Ficus aurea	5	1	1	0.2 ± 0.09	5	1415	283.0 ± 112.92	2.50 (0.037)
Leptospermum rotundifolium	4	1	1	0.3 ± 0.13	4	1948	487.0 ± 114.02	4.27 (0.005)
Mosiera longipes	7	1	1	0.1 ± 0.05	7	2311	330.1 ± 19.19	17.2 (<0.001)
Psidium guajava	5	1	1	0.2 ± 0.09	5	1312	262.4 ± 66.53	3.94 (0.004)
Syzygium jambos	4	1	1	0.3 ± 0.13	3	969	242.3 ± 115.80	2.50 (0.055)
Tibouchina granulosa	8	1	1	0.1 ± 0.04	8	1874	234.3 ± 37.82	6.19 (<0.001)

[a]If plant species are not listed, then no eggs were oviposited on them. Means ± S.E. are presented along with results of a *t*-test comparing means between each test plant species and corresponding *M. quinquenervia* controls.

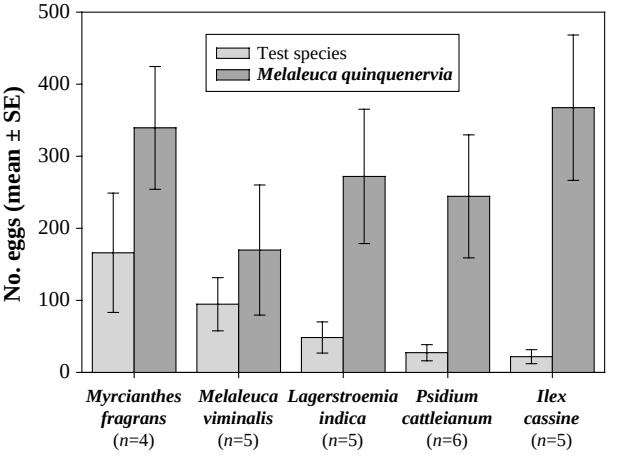

Figure 2. Mean number of eggs (±SE) observed on the five nontarget plant species most attractive to *Lophodiplosis trifida* for oviposition as compared to *Melaleuca quinquenervia* controls.

though their corresponding controls may have failed. The distribution of adults emerging from galls on *M. quinquenervia* controls was as follows: 105 of 117 (90%) galled plants produced ≥19 new adults; 102 of 105 (97%) produced ≥30; 85 of 105 (83%) produced ≥ 60; and 57 of 105 (67%) produced ≥90.

The reliability of the controls in the development tests was very strong. *Lophodiplosis trifida* completed its life cycle on 96% of the usable *M. quinquenervia* controls (101 out of 105). The remaining four plants would also have produced adults had the tests continued beyond 6 weeks.

Lophodiplosis trifida did not complete development on any nontarget species. Examination of the 6-week-old swollen *M. viminalis* stems revealed the presence of small, unilocular galls (Figure 1e) that contained larvae similar in size to the first instar larvae from *M. quinquenervia* (Figure 1f). An empty chamber, smaller than a typical mature chamber on *M. quinquenervia,* was found once on *M. viminalis*. This observation suggested

Table 4. Results of examinations of plants exposed to *Lophodiplosis trifida* in no choice development tests.

Observations	*Melaleuca quinquenervia*	*Melaleuca viminalis*
Macroscopic examination		
% Plants galled ($N=7$)	100	57
% Plants with 3 galled stems	100	43
% Plants with 2 galled stems	–	14
Microscopic examination		
Plants galled (N)	7	4
Chambers/plant ($\bar{X}\pm$S.E.)	206.1±56.70	181.5+67.77[b]
Range	28–405	0–326
Developmental stages observed	Larvae, pupae, empty chambers[a]	Larvae

[a]If an empty mature gall chamber was found with an exit hole, it was counted as a chamber from which an adult had emerged. [b]In a paired t-test, $t=-0.271$, df $=0.793$, $P=0.793$. The power of the performed test, 0.296 was below the desired power of 0.800.

that an abnormally small adult may have emerged, but no pupae or adults were observed during dissections of hundreds of other *M. viminalis* chambers.

It is evident that *L. trifida* initiated gall development on *M. viminalis* (Table 4, macroscopic examination). However, the disparity in the mean number of chambers/plant (although differences were not significant: paired *t*-test, Table 4, microscopic examination) along with the reduced frequency of galls suggests a lesser probability of *M. viminalis* plants becoming galled. In addition, with one possible exception, no larvae completed development on *M. viminalis*. As gall formation and insect development are codependent, the larvae found in *M. viminalis* died and gall formation did not progress when the *M. viminalis* tissue failed to sustain them.

Abortive gall development on *M. viminalis* caused minor cosmetic damage consisting of swollen tissue where galls were initiated. Plants were not held to determine the effect of the swollen tissue on further growth but Purcell and Brown (personal communication) observed that affected stems of *M. viminalis* plants in Australia grew normally.

In summary, *L. trifida* is not selective in its oviposition behaviour when caged in a laboratory environment. However, it unquestionably and consistently attacks, galls, and completes development only on *M. quinquenervia* and was unable to do so on any nontarget species tested. This insect is clearly host specific.

Moreover, *L. trifida* causes unalterable damage to *M. quinquenervia* that prevents further growth. For example, after 30 days exposure to *L. trifida* in an Australian greenhouse study, differences in height of 45 *M. quinquenervia* seedlings was reduced significantly when compared to 45 plants not exposed (Purcell, personal communication). In a 10-month Florida quarantine study of two young plants, one exposed to 15 *L. trifida* females and one not, obvious differences in growth were apparent after 5 months (Figure 1h).

Due to its host specificity and propensity to suppress *M. quinquenervia* growth and reproduction, we have proposed to introduce the stem-gall fly, *L. trifida*, from Queensland, Australia to south Florida. It seems less fastidious in terms of preferred tissues than *F. turneri*, so it should establish more readily. A petition to release *L. trifida* was submitted to the Technical Advisory Group (TAG) of the USDA (2008) Animal and Plant Health Inspection Service (APHIS) in May 2007. Notification of TAG's recommendation for its release was received in December 2007.

Acknowledgements

We thank J.A. Lollis for his painstaking assistance in the testing process, growing a steady supply of *M. quinquenervia* plants, and maintaining test plants; J.K. Balciunas, M.F. Purcell, D.W. Burrows, J.R. Makinson, and B.T. Brown at the USDA ARS ABCL/CSIRO Entomology, Brisbane, Australia, for their discovery of *L. trifida*, and providing preliminary testing and information on the biology of *L. trifida*; R.J. Gagné, USDA ARS, Systematic Entomology Laboratory, Washington, DC, for describing *L. trifida*, confirming identification of imported *L. trifida*, and answering various questions about *L. trifida* biology; L.A. Craven, Australian National Herbarium, Canberra, for confirming identification of *Melaleuca* spp. and answering botanical questions; S.E. White and H. Furlong, USDA ARS Center for Medical and Veterinary Entomology (CMAVE), Gainesville, FL, for routinely screening *L. trifida* for pathogens; R.E. Weaver, FDACS DPI, Gainesville, FL, for confirming identification of local plants; G.J. Steck and J.D. Stanley, FDACS DPI, for photographing *L. trifida* larvae. This research was supported in part by the South Florida Water Management District and by the USDA ARS.

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